ON THE USE OF ANHYDROUS SODIUM SULPHITE IN THE PREPARATION OF ENDO'S MEDIUM, TOGETHER WITH A NOTE ON THE PREPARATION OF ANHYDROUS SODIUM SULPHITE AND ITS STABILITY UNDER ORDINARY CONDITIONS.*

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As is well known, Endo's medium is frequently employed as a means whereby to separate the typhoid organism from the colon bacillus, preliminary to further identification. According to Endo[1] this medium is prepared as follows:

Five hundred gm. ground beef, 10 gm. peptone, 5 gm. sodium chloride, and 30 gm. agar are added to one liter of water, the whole is well cooked, filtered, neutralized, and 10 c.c. of a 10 per cent soda solution added in order to make it alkaline, after which 10 gm. of c.p. milk sugar and 5 c.c. of an alcoholic solution of fuchsin are added as the result of which the medium becomes colored red. Then 25 c.c. of a 10 per cent solution of sodium sulphite are added, whereby the medium is gradually decolorized. It becomes entirely colorless, however, only after the agar has solidified. After the introduction of the medium into petri dishes it is sterilized for 30 minutes in a steam sterilizer. The author remarks first that the milk sugar must be chemically pure for the reason that the commercial product often contains cane sugar, from which the typhoid bacillus produces acids in consequence of which it becomes difficult to distinguish it from the colon bacillus; second, the sodium sulphite solution must be preserved in a well closed flask or should be prepared fresh for use; third, the alcoholic fuchsin solution must be previously filtered; fourth, the medium should be preserved in the dark, since as the result of the action of light it gradually acquires a red color. Petri plates prepared by the use of this medium are quite colorless and transparent. The colonies of the colon bacillus after 24 hours become deep red and show a greenish metallic sheen on the surface; on the other hand, the colonies of the typhoid bacillus are transparent and colorless, like minute drops of water.

Klinger[2] gives essentially the same directions for the preparation of this medium. Unfortunately neither of these authors gives any information regarding the particular form of sodium sulphite employed in the preparation of the medium. The results obtained by various workers in the Division of Pathology and Bacteriology of this labora-

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[1] *Centralbl. f. Bakt.*, Orig., 1903–4, 35, p. 109.

[2] *Arb. a. d. kais. Gesundheitsamt.*, 1906, 24, p. 52.

tory point to the fact that the ordinary hydrated modification of sodium sulphite, $Na_2SO_3 \cdot 7H_2O$, is the form ordinarily employed in the preparation of the medium and is the variety of the compound originally employed by Endo and also by Klinger. In this connection considerable difficulty has been experienced from time to time by the bacteriologists of the Hygienic Laboratory in obtaining a perfectly reliable and satisfactory Endo medium. For these failures two things seem to be primarily responsible: (1) impure lactose and (2) impure sodium sulphite. The first difficulty has been overcome by the use of Kahlbaum's c.p. lactose, and upon Kastle's suggestion the second difficulty has been overcome by the use of pure anhydrous sodium sulphite in half the quantity originally employed by Endo.

In this and other connections, considerable work has been done in this laboratory, during the past several years, on the sulphites, and a considerable number of samples of sodium sulphite, both of our own preparation and those obtained from chemical manufacturers, have been analyzed.

Thus a sample of heptahydrate of sodium sulphite ($Na_2SO_3 \cdot 7H_2O$) obtained from one of the most careful and reliable firms in the country gave the following results on analysis:

	Found (Per cent)
Sodium Sulphite	22.05
Sodium Sulphate	25.00
Water (loss on drying in vacuo at 100° C.)	52.50
	99.55

On the other hand, a fresh sample of the heptahydrate of sodium sulphite prepared in this laboratory gave the following numbers:

	Found (Per cent)	Theory for $Na_2SO_3 \cdot 7H_2O$ (Per cent)
Sodium Sulphite	47.58	49.99
Water	52.11	50.01
	99.69	100.00

After standing for six months in a glass-stoppered bottle, under ordinary conditions, the bottle having been opened a few times during this interval in order to remove small amounts of the salt, the latter sample of sodium sulphite was found to contain only 40.84 per cent of sodium sulphite, Na_2SO_3. Hence under these conditions this sample of the

salt had lost approximately one-fifth of its total available sulphite. On the other hand, it has recently been pointed out by Hartley and Barrett[1] that anhydrous sodium sulphite, Na_2SO_3, is stable so long as it is kept dry, whereas they also have found that the hydrated salt, $Na_2SO_3 \cdot 7H_2O$, oxidizes very readily in the air. And in this connection these authors have devised a method for the preparation and drying of anhydrous sodium sulphite out of contact with the air to which reference will be made in a subsequent part of this communication. It therefore occurred to one of us (Kastle) that in the preparation of the Endo medium much more regular and uniform results could be secured by the use of anhydrous sodium sulphite in half the quantities recommended by Endo and Klinger, the reason for taking half the quantity of sulphite being that the anhydrous salt contains almost exactly twice as much of the compound Na_2SO_3 as ordinary sodium sulphite crystals, viz., the heptahydrate, $Na_2SO_3 \cdot 7H_2O$ (see theory for $Na_2SO_3 \cdot 7H_2O$, p. 620). The preparation of the Endo medium with anhydrous sodium sulphite and Kahlbaum's c.p. lactose was first carried out by Dr. Frost in the Division of Pathology and Bacteriology of this laboratory. One lot of the medium was prepared according to Endo's directions, using a 10 per cent solution of anhydrous sodium sulphite, and a second lot with a 5 per cent solution of the anhydrous sulphite. The typhoid and colon bacilli were found to grow upon both, the colon colonies becoming deep purplish red with a greenish metallic sheen, whereas the typhoid colonies were colorless. Dr. Frost found, however, that the growths of the two organisms were more typical when grown on the medium made up with the 5 per cent solution of anhydrous sodium sulphite. This also goes to show, as was suspected, that both Endo and Klinger used a 10 per cent solution of ordinary sodium sulphite crystals ($Na_2SO_3 \cdot 7H_2O$) in the preparation of the medium. In connection with the investigation of typhoid fever now being carried out in this laboratory large amounts of the Endo medium are used and in no instance has any difficulty been experienced in its preparation since these suggestions were made regarding the use of anhydrous sodium sulphite and chemically pure lactose for its preparation.

[1] *Jour. Chem. Soc.*, Trans., 1909, 95, pp. 1178–85.

The method now employed in the preparation of the medium is as follows:[1]

Ten gm. of Liebig's extract of beef, 10 gm. of peptone, and 5 gm. of sodium chloride are added to one liter of distilled water. This mixture is then heated until these substances have dissolved. It is then allowed to cool, and 40 gm. of powdered agar are placed on the surface. When this has settled, the mixture in a beaker is placed in an Arnold sterilizer, covered with paper, and allowed to cook for three hours. The solution is now made neutral to litmus paper with sodium carbonate. It is then filtered through cotton on a perforated funnel (Buchner filter), with the aid of the pump, or allowed to settle while slowly cooling, rejecting the turbid bottom portion. To the filtered solution 10 c.c. of sterile 10 per cent sodium carbonate solution are added. This medium may be conveniently preserved in quantities of 100, 200, and 400 c.c. in flasks, the flasks being considerably larger than is required for these quantities, in order to provide room for the other ingredients. In this way the medium can be stored until required for use, when the agar is melted and the other ingredients added as follows:

To each liter of the above medium add 10 gm. of c.p. lactose and 5 c.c. of freshly filtered alcoholic fuchsin, prepared by shaking 10 gm. of fuchsin (not acid fuchsin) with 100 c.c. of 96 per cent alcohol, allowing to stand 24 hours, decanting the supernatant fluid, and filtering this each time immediately before use. The medium is then vigorously shaken and placed unstoppered in the sterilizer for from five to ten minutes in order to allow the foam to settle, after which 25 c.c. of a freshly prepared, sterile, 5 per cent solution of anhydrous sodium sulphite are added. This is mixt into the medium by gently rotating the flask in order to avoid foaming. The Endo medium thus prepared is then sterilized for a few minutes in the Arnold sterilizer and poured into the petri dishes while it is steaming hot. After cooling, the medium should be nearly colorless to transmitted light, and rose or flesh colored to reflected light. The lactose, fuchsin, and sodium sulphite solutions must be added to the melted agar just before it is to be used. The plates are flown, and allowed to stand 20 minutes uncovered in the incubator in order to remove water of condensation and to obtain a good surface. Organisms which split lactose restore the red color of the fuchsin and appear as deep red, sharply defined, opaque colonies with a greenish metallic sheen; the typhoid organism produces smaller transparent colonies, resembling small drops of water.

The anhydrous sodium sulphite used by the bacteriologists of the Hygienic Laboratory in their recent work with the Endo medium was prepared by Elvove, using a modification of the method of Hartley and Barrett, which is described in the latter part of this communication, and which on analysis by the direct method of Giles and Shearer[2] was found to contain 99 per cent of anyhdrous sodium sulphite, Na_2SO_3.

In this connection it seemed of interest to obtain some data as to

[1] See Report No. 3 on the "Origin and Prevalence of Typhoid Fever in the District of Columbia, 1909," *Bulletin No.*— of the Hygienic Laboratory, P. H. and M. H. S., pp. — (in press).

[2] *Jour. Soc. Chem. Ind.*, 3, p. 197; 4, p. 303.

the purity of a number of specimens of commercial anhydrous sodium sulphite, as well as additional data relative to the stability of commercial and other specimens of this salt under ordinary conditions. This has been done by Elvove.

Samples of anhydrous sodium sulphite were obtained from a number of chemical firms of recognized standing, including the well known firms of C. A. F. Kalhbaum, Merck and Co., and the J. T. Baker Chemical Co. As soon as these were received their purity was compared with the specimen of the salt which had been prepared by Elvove, after which they were kept for various lengths of time and analyzed at the end of each of the periods indicated, by the method of Giles and Shearer. The results of the analyses of the several samples are given in Table 1.

TABLE 1.

COMPARISON OF THE PURITY OF SAMPLES OF ANHYDROUS SODIUM SULPHITE OBTAINED FROM VARIOUS SOURCES.

Number of Sample	Amount Taken for Analysis	N/10 Iodine Required	Percentage Purity
	gm.	c.c.	
1 (Commercial)	0.1260	18.25	91.25
2 "	0.1260	18.75	93.75
3 "	0.1260	19.20	96.00
4 "	0.1260	19.30	96.50
5 "	0.1260	19.30	96.50
6 "	0.1260	19.45	97.25
7 (Prepared by Elvove's method)	0.1260	19.80	99.00
8 (Prepared and analyzed by Hartley and Barrett, using an indirect method of analysis)			99.87*

* Another analysis by these authors showed the presence of 63.23 per cent SO_3 or a purity of 90.62 per cent. However, on account of the indirect method of analysis (oxidation to sulphate and determining total sulphate) used by Hartley and Barrett, these figures do not necessarily show that their product actually contained this amount of anhydrous sodium sulphite, Na_2SO_3, and hence was purer than that obtained by the modified method which showed on analysis by the direct method of Giles and Shearer the actual presence of only 99 per cent of anhydrous sodium sulphite. Thus Hartley and Barrett found that 0.6674 gm. of their salt gave 1.2334 gm. $BaSO_4$. If this amount of their salt had actually contained a sulphate impurity of 0.62 per cent (the difference between the analyses compared) it would have altered the weight of the $BaSO_4$ to the extent of only 0.0009 gm., i. e., only about 0.07 per cent. In other words, a sulphate impurity of 0.62 per cent would be concealed by as little as 0.07 per cent error in the gravimetric sulphate determination. On the other hand, by using the direct method of Giles and Shearer the impurity present is not minimized; and in a case as just mentioned would manifest itself by requiring about 0.65 c.c. of N/10 iodine less, which is an amount beyond experimental error. Further, the figures given for the percentage purity of the product obtained by the modified method simply show the purity of the sample which was used in this work and do not necessarily represent the highest purity obtainable by the modified method. As compared with the original method of Hartley and Barrett, there is also *a priori* no reason why the modified method should yield a salt of less purity, since all of Hartley and Barrett's precautions to avoid oxidation are included in the modified method; while there is reason why the latter might be expected to yield a product of even higher purity than the Hartley and Barrett method, since even the preliminary saturation with the SO, is, in the modified method, conducted out of contact with the air.

These results show clearly that the specimen of anhydrous sodium

sulphite obtained by Elvove by a modification of Hartley and Barrett's method is of greater purity than the best anhydrous sodium sulphite which we have thus far been able to obtain from chemical manufacturers. It is quite likely, however, that any of these lots of commercial anhydrous sodium sulphite are sufficiently pure for the preparation of a satisfactory and reliable Endo medium.

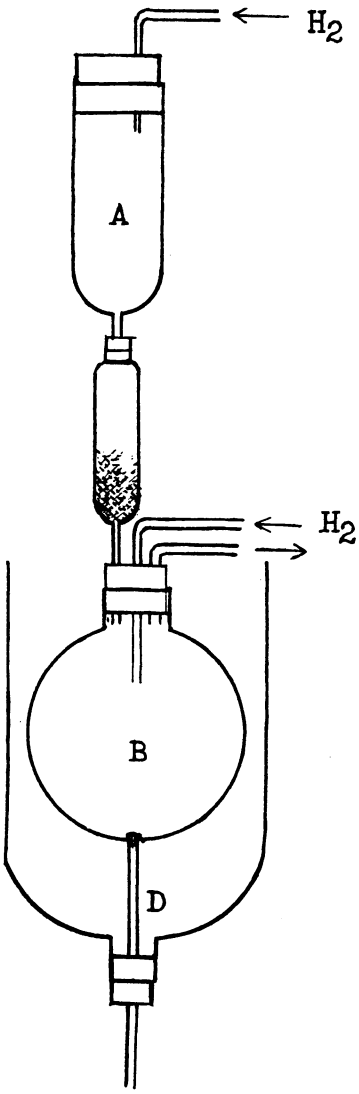

FIG. 1.—Diagram of Hartley and Barrett's apparatus.

For the benefit of those who may wish to prepare pure anhydrous sodium sulphite, however, we will give a brief description of the method recently proposed by Hartley and Barrett for this purpose, and also the modification thereof employed by Elvove. Hartley and Barrett's method is as follows:

Forty gm. of Merck's pure sodium carbonate were dissolved in 120 gm. of air-free water, and a current of sulfur dioxide passed into the solution until the gain in weight showed that it was converted into sodium hydrogen sulphite; an equal quantity of sodium carbonate solution was then added, and the solution was quickly transferred to the vessel *A* (Fig. 1), containing hydrogen, whence it was filtered through glass wool into the vessel *B*, which was surrounded by a bath of brine kept at a temperature of over 100° by blowing in steam. As the anhydrous salt is less soluble than the hydrated form above 22°, it crystallizes when the temperature of the solution is raised and the yield is increased by evaporating the solution in a stream of hydrogen. After some hours, the solution was drawn off by the tube *D*, the crystals being stopped by a small glass plug. The crystals were first washed with a mixture of alcohol and water, when with alcohol, and finally dried, all the operations being carried on in an atmosphere of hydrogen. The yield varied from 11 to 24 gm.

While, therefore, this method gave an excellent product, it required a special apparatus and also a supply of superheated steam for keeping the brine bath at a temperature of above 100° C.; the yield is small, the minimum being 11.6[1] per cent and the average only 18.4 per cent of what it should yield theoretically, and the time required

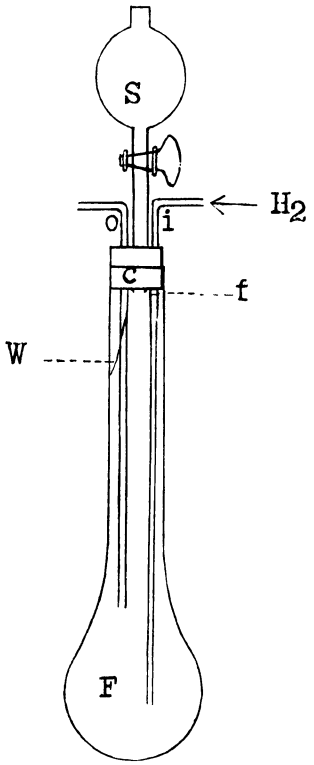

FIG. 2.—Modified arrangement.

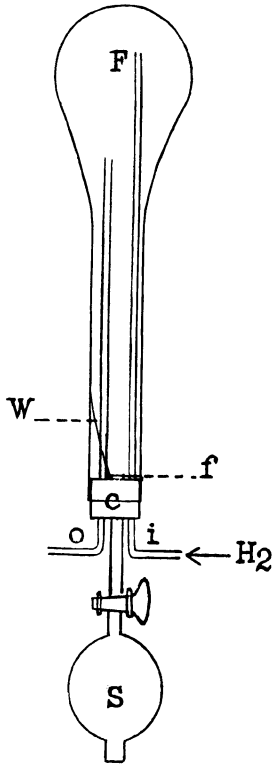

FIG. 3.—Modified arrangement (in position for draining off liquid).

is comparatively long. Inasmuch, however, as it appeared probable that the chief, if not the only reason, for effecting the evaporation by heating with steam instead of over a free flame is that the special apparatus required does not well adapt itself to the latter mode of heating, it therefore occurred to one of us (Elvove) that if we could

[1] These numbers are based on the assumption that the sodium carbonate referred to by Hartley and Barrett means the anhydrous sodium carbonate, since, as evaporation must be resorted to for concentrating the solution, they probably used as strong a solution as is feasible in the first place, making this solution from the anhydrous carbonate, or at least the monohydrate. Even had they used a hydrate of sodium carbonate, their yield would still have been smaller than that obtained in much less time by the modified method here described.

devise an arrangement which would permit of all the operations being conducted in an atmosphere of hydrogen and which could also conveniently be heated over a free flame, the necessity for a supply of superheated steam could be avoided and probably also one could obtain an increased yield in a shorter time. As a result, Elvove devised the following simple arrangement, which is illustrated diagrammatically in Figs. 2 and 3, and which was found to answer this purpose very satisfactorily.

F is an ordinary Kjeldahl flask, *S* is an ordinary globular separatory funnel. These are connected together by means of a well fitting cork, *c*, through which pass the hydrogen inlet tube, *i*, and outlet tube, *o*. The tubular end of the separatory funnel just passes through the cork, its end being nearly flush with the inner end surface of the cork, and has over it a muslin filter,[1] *f*. *W* is a piece of platinum wire one end of which is attached to the tubular end of the separatory funnel by insertion between the latter and the surrounding cork, while the other end touches the side of the flask, and which serves to lead the liquid from the separatory funnel into the flask and thus avoid the tendency of the liquid to flow partly down the outlet tube and escape. The connection of the apparatus to the hydrogen supply is made by means of soft rubber tubing (not shown in the figures) of sufficient length as to permit of the whole apparatus being inverted or moved about.

MODIFIED METHOD FOR PREPARING ANHYDROUS SODIUM SULPHITE.

Eighty gm. of pure anhydrous sodium carbonate were dissolved in freshly boiled water, using sufficient of the latter to make the total volume 240 c.c. Half of this was then transferred to the flask, *F* (Fig. 2), and sulfur dioxide passed into it to saturation, leading the exit gases into a tube containing a dilute aqueous solution of crystal violet which, in addition to serving as a liquid seal in preventing the entrance of atmospheric air into the flask, aids also in determining when the solution in the flask has become saturated with the sulfur dioxide. When the disappearance of the color in the crystal violet solution, as well as the odor of the exit gases, indicated that the liquid in the flask had become saturated with the sulfur dioxide, the supply of the latter was shut off and the inlet tube of the apparatus connected with the hydrogen supply, the inlet tube being raised above the level of the liquid in the flask, which now presented a perfectly clear solution (the crystalline precipitate which formed at first having been completely redissolved on further addition of the SO_2), and the hydrogen allowed to pass through. The solution was then warmed for a few minutes, when the remaining half of the sodium carbonate solution was added through the separatory tunnel,[2] *S* (Fig. 2). It was then boiled over a free flame until it was reduced in bulk to about one-third of the original volume, this reduction in volume being

[1] A convenient way of attaching the muslin filter consists in selecting a thin piece of soft muslin and cutting from it a narrow strip whose length is a little more than double the length of the cork and pressing its central part, by means of a suitable rod, completely into the opening in the cork which is intended for the tubular end of the separatory funnel and then gently work the latter in place.

[2] In using the separatory funnel as an inlet, the passage of the liquid may be hastened by connecting with a piece of rubber tubing and applying some pressure.

effected in less than one hour. By gently inverting the whole apparatus while still hot the crystals of Na_2SO_3 were separated from the mother liquor, running the latter out through the muslin filter, *f* (Fig. 2). The crystals thus obtained were then washed twice with a mixture of equal volumes of water and alcohol, using 40 c.c. of the mixture each time; followed by three washings with absolute alcohol, using 20 c.c. of the latter for each washing. The whole apparatus up to the mouth of the flask was then immersed in an air oven[1] and heated to 110–120° C. for one hour, during which time the passage of the current of hydrogen remained uninterrupted. In this way 70 gm. of anhydrous sodium sulphite, or about 73.6 per cent of the theory, were obtained. An analysis by the direct method of Giles and Shearer showed the product thus obtained to contain 99 per cent of Na_2SO_3.

The chief advantages of this method over the original method of Hartley and Barrett may be summed up as follows:

1. No special apparatus is required, and no supply of steam is necessary.

2. The whole operation, including the saturation of the sodium carbonate with sulfur dioxide and subsequent drying of the salt, can be carried out in one apparatus.

3. The time required for evaporation is reduced to less than one hour.

4. The yield is increased from 11.6 to 25.3 per cent, to over 73.0 per cent.

THE STABILITY OF ANHYDROUS SODIUM SULPHITE UNDER ORDINARY CONDITIONS.

In order to obtain additional data relative to the stability of anhydrous sodium sulphite under ordinary conditions, a number of commercial samples of this salt, as well as that prepared by the method above described, were placed in paraffin-sealed bottles and also in ordinary glass-stoppered bottles which were kept without any special precautions on a table in the laboratory, and which during the time that samples were being drawn for analysis remained entirely exposed to the atmosphere of the laboratory. The results obtained are given in Tables 2 and 3.

As may be seen from the results given in Tables 2 and 3, anhydrous sodium sulphite is quite stable even when kept in glass-stoppered

[1] A convenient air oven for this purpose, in the absence of another better suited for it, may be made by cutting off a piece of sheet iron stove pipe to the proper length and placing a piece of sheet metal under the lower opening to serve as a bottom, while a similar piece of sheet metal, provided with a central opening to fit the neck of the flask and to allow the insertion of a thermometer and divided diametrically into halves across the opening, may serve as a top covering.

TABLE 2.

STABILITY OF ANHYDROUS SODIUM SULPHITE KEPT IN PARAFFIN-SEALED BOTTLES.

Number of Sample	Length of Time Kept	Amount Taken for Analysis	N/10 Iodine Required	Percentage Purity
	days	gm.	c.c.	
1	1	0.1260	18.25	91.25
1	15	0.1260	18.25	91.25
1	30	0.1260	18.20	91.00
2	1	0.1260	18.75	93.75
2	15	0.1260	18.70	93.50
2	30	0.1260	18.75	93.75
3	1	0.1260	19.20	96.00
3	15	0.1260	19.05	95.25
3	30	0.1260	18.95	94.75
4	1	0.1260	19.30	96.50
4	15	0.1260	19.30	96.50
4	30	0.1260	19.20	96.00
5	1	0.1260	19.30	96.50
5	15	0.1260	19.30	96.50
5	30	0.1260	19.25	96.25
6	1	0.1260	19.45	97.25
6	15	0.1260	19.30	96.00
6	30	0.1260	19.30	96.00
7	1	0.1260	19.80	99.00
7	15	0.1260	19.70	98.50
7	30	0.1260	19.70	98.50

TABLE 3.

STABILITY OF ANHYDROUS SODIUM SULPHITE KEPT IN GLASS-STOPPERED BOTTLES.

Number of Sample	Length of Time Kept	Amount Taken for Analysis	N/10 Iodine Required	Percentage Purity
	days	gm.	c.c.	
1	1	0.1260	18.25	91.25
1	3	0.1260	18.20	91.00
1	7	0.1260	18.25	91.25
1	15	0.1260	18.25	91.25
1	28	0.1260	18.20	91.00
2	1	0.1260	18.70	93.50
2	3	0.1260	18.70	93.50
2	7	0.1260	18.75	93.75
2	15	0.1260	18.70	93.50
2	28	0.1260	18.75	93.75
3	1	0.1260	19.10	95.50
3	3	0.1260	18.95	94.75
3	7	0.1260	18.90	94.50
3	15	0.1260	18.90	94.50
3	28	0.1260	18.95	94.75
4	1	0.1260	19.10	95.50
4	3	0.1260	19.10	95.50
4	7	0.1260	19.00	95.00
4	15	0.1260	19.00	95.00
4	28	0.1260	19.05	95.25
5	1	0.1260	19.30	96.50
5	3	0.1260	19.25	96.25
5	7	0.1260	19.20	96.00
5	15	0.1260	19.20	96.00
5	28	0.1260	19.25	96.25
6	1	0.1260	19.20	96.00
6	3	0.1260	19.25	96.25
6	7	0.1260	19.15	95.75
6	15	0.1260	19.20	96.00
6	28	0.1260	19.20	96.00
7	1	0.1260	19.80	99.00
7	3	0.1260	19.80	99.00
7	7	0.1260	19.75	98.75
7	15	0.1260	19.70	98.50
7	28	0.1260	19.75	98.75

bottles under ordinary laboratory conditions, thus confirming the experience of Hartley and Barrett[1] in this respect, who found that anhydrous sodium sulphite is stable so long as it is kept dry.

SUMMARY.

On account of its greater purity and stability under ordinary conditions, anhydrous sodium sulphite may be employed to advantage in the preparation of Endo's medium.

An improved method (Elvove's) for the preparation of pure anhydrous sodium sulphite is described.

Anhydrous sodium sulphite has been found to be quite stable under ordinary conditions, especially when kept dry.

A number of chemical manufacturers supply anhydrous sodium sulphite of sufficient purity for the preparation of a satisfactory Endo medium.

[1] *Loc. cit.*, p. 1179.